I. Prostate Cancer

Prostate cancer has emerged as a major public health concern. The lifetime risk for developing cancer of the prostate (CaP) in American males is one in five. Although there is no known cause for CaP, there are several factors that may increase the risk of CaP development, including genetics, race, age, or diet (1-3). It has been reported that certain chromosomal regions contain risk factors for CaP, and consistent with this, an individual's risk more than doubles if a close relative has CaP, with two relatives, it increases fivefold, and with three relatives, risk is virtually 100%. Blacks are twice as likely to be diagnosed with CaP and have twice the mortality rate than that of whites. Asian men have the lowest incidence of CaP, but upon emigration to the US, their rates rise to almost that of whites (4). Genetics, testosterone levels, and diet are believed to play a part in these racial differences. In addition, risk of CaP increases with age. Men over 65 years of age are at the highest risk; however, 25% of all reported cases are diagnosed under the age of 65.

CaP incidence rates increased 141.8% between 1973 and 1994, and in 1998, new cases totaled over 180,000. In 1999, it is estimated that 41,000 men will die from CaP in the United States (5). This cancer continues to be the most frequently diagnosed malignancy, aside from skin cancers, representing 29% of all new cancer cases in US men, and the mortality rate is second only to heart disease in this group.

According to the National Cancer Institute, as measured by lost wages, productivity, and medical costs, CaP costs up to $15 billion annually, and currently, the federal government spends 50 times more in patient care than in research to find a cure.

II. Currently Available Treatments

CaP can be a difficult disease to detect and treat. It is a multi-focal disease, i.e. there is often more than one focus of malignant cells in the organ, and often varying stages of differentiation exist between individual foci. Treatment options are limited to surgery or radiation therapy for localized disease. Surgical treatment (prostatectomy) is most common among younger, healthier patients in whom gross metastatic events have been ruled out; however, this treatment can have side effects that severely compromise the patient's quality of life such as incontinence and sexual dysfunction. Radiation therapy is less invasive and involves either the directing of x-rays into the pelvic area, or implanting radioactive pellets into the prostate. However, all forms of radiotherapy are associated with complications, including acute cystitis, prostatitis, enteritis, and urinary/sexual dysfunction.

In patients with metastatic CaP, androgen ablation is palliative therapy that serves to reduce tumor burden and maximize patient longevity. This is achieved by medical or surgical castration. However, hormonal therapy can have significant side effects, Not all patients can tolerate the drugs, and almost all lose sexual function. Several hormonal therapies exist to eliminate androgens. Surgical removal of the testis will reduce testosterone levels to 5-10%, and when combined with bilateral adrenalectomy or treatment with aminoglutethimide, testosterone levels become undetectable. Administration of diethylstilbestrol, an estrogen, has been useful, although it is associated with severe cardiovascular side effects, Currently in use are the luteinizing hormones releasing hormone (LHRH) agonists, These are powerful stimulators of the hypothalamus, causing it to release luteinizing hormone (LH), which stimulates the

production of testosterone. In the presence of LHRH agonists, the body fails to make normal LHRH, there is no release of LH, and serum levels of testosterone falls to castrate levels. To further inhibit the action of androgen, non-steroidal antiandrogens are used in conjunction with LHRH agonists. The mechanism of non-steroidal antiandrogens is not completely understood, but they block dihydrotestosterone, the active form of testosterone, from stimulating protein synthesis in prostate cells. Although these forms of hormonal therapy will eliminate hormone-sensitive cells and reduce tumor burden by approximately 80%, the remaining hormone-resistant disease will continue to proliferate and eventually result in the death of the patient. No effective treatment for hormone-refractory prostate cancer is available. Because of prostate cancer's obvious medical ramifications, there is a great need for the development of an effective treatment.

III. Immunotherapy

In the early twentieth century Coley used bacterial infections to initiate an antitumor response (6). Although not understood, these observations formed the basis for the supposition that immuno-adjuvant therapy could override tumor escape mechanisms and induce an antitumor response. The general promise of this hypothesis failed to materialize into clinically effective therapy, although adjuvant BCG therapy for bladder cancer emerged as an effective treatment regimen (7). The overall lack of success of these adjuvant immunotherapy regimens lead to doubt about the ability of the immune response to effectively eliminate tumors.

Rosenberg and associates revitalized interest in immunotherapy with their work on LAK and TIL (8-10). These experiments demonstrated the presence of immune cells that could be activated *in vitro*. The *in vitro* activated cells mediated antitumor activity on adoptive transfer into tumor-bearing hosts (8). Again, the therapeutic efficacy of clinical trials fell short of expectations. However, the studies clearly demonstrated the ability of immune cells to eliminate tumors previously considered to be resistant to immune effector mechanisms (10).

Gene therapy studies confirmed the hypothesis that most theoretically "non-immunogenic" tumors were indeed immunogenic (11-18). These studies demonstrated that expression of cytokines or co-stimulatory molecules in sufficient quantities at the tumor site induced an antitumor response. Neither the systemic administration of cytokines nor the production of cytokines by transfected cells at sites distant from the tumor induced an antitumor response (14-18). Only rarely did cytokine gene therapy induce regression of existing tumors at secondary sites, and this occurred only in the early growth stages (12). In some systems regression of small tumor burdens could be induced by multiple immunizations with lL2-transfected tumor cells (18).

The use of microbial vectors to carry foreign proteins as vaccines in cancer therapy has been documented in a number of experimental systems (reviewed in 19). A few examples of this research include Rosenberg's group use of replication-defective adenovirus vaccines to elicit anti-tumor immunity in mouse models of colon carcinoma and melanoma (20-22). Herlyn and colleagues have effectively demonstrated the *use* of adenoviral vaccines in the treatment of a mouse colon carcinoma (23). From these data and those of others, it is clear that recombinant microbial vaccines appear to lead to more effective antigen presentation in the tumor cell. This may be a direct result of

tumor antigen synthesis within the antigen-presenting cell (APC) or may be a consequence of antigen expression outside the suppressive effects of the tumor (19).

Several possible target antigens for immunotherapy that are unique to the prostate have been identified. These include PSA (hK3), human glandular kallikrein II (hK2), prostate-specific membrane antigen (PSMA), and prostatic acid phosphatase. These antigens are produced by normal prostate epithelial cells and most prostate cancer cells, whether androgen-dependent or androgen-independent. Our laboratory as well as the laboratories of other investigators have demonstrated the presence of both antibody production and T cell reactivity to PSA (24-31 & unpublished observations). Since these are normal antigens that activate T cell responses *in vitro* it is probable that the absence of an immune response to the antigens is associated with the expression of peripheral tolerance in the form of anergy. Thus, studies have been proposed that would develop viral and bacterial vectors expressing these antigens in order to abrogate the anergic state and induce antitumor immunity.

The development of immunotherapy protocols for the treatment of human prostate cancer using PSA is dependent on the ability to overcome immune tolerance to the antigen. Studies in numerous autoimmune and tolerance models demonstrate that tolerance mechanisms can be abrogated and that the resulting immune response is tissue destructive. The hypothesis that forms the foundation for research used as the basis for this clinical trial is that activation of the immune response to prostate-associated antigens will initiate an antitumor response.